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MRID No.: 436491-09

DATA EVALUATION RECORD
§ 72-2 - ACUTE LC_{50} TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** Didecyldimethylammonium-carbonate (DDA Carbonate) — PC Code No.: 069208
2. **TEST MATERIAL:** DDA Carbonate — Purity: 45.9%
   ^{14}C-DDA Chloride — 96.9%
3. **CITATION:**
   Author: Maura K. Collins
   Title: Didecyldimethylammoniumcarbonate (DDA Carbonate) - Evaluation in a Static Acute Toxicity Test with *Daphnia magna*
   Study Completion Date: June 20, 1994
   Laboratory: Springborn Laboratories, Inc., Wareham, MA
   Sponsor: Lonza Inc., Fair Lawn, NJ
   Laboratory Report ID: 94-5-5257
   MRID No.: 436491-09
   DP Barcode: D218362
4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.,
   Signature: [signature] **Date:** 11/30/95
   APPROVED BY: Mark Mossler, M.S., Toxicologist, KBN Engineering and Applied Sciences, Inc.,
   Signature: [signature] **Date:** 11/30/95
5. **APPROVED BY:**
   Signature: [signature] Henry T. Craven 2/6/96 **Date:** 2-6-96
6. **STUDY PARAMETERS:**
   Age of Test Organism: ≤24 hours
   Definitive Test Duration: 48 hours
   Study Method: Static
   Type of Concentrations: Mean measured
7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity study using freshwater invertebrates. The LC_{50} value of 66 ppb ai classifies didecyldimethylammoniumcarbonate as very highly toxic to *Daphnia magna*.

Results Synopsis

EC_{50}:	66 ppb ai	95% C.I.:	33-100 ppb ai
NOEC:	33 ppb ai	Probit Slope:	N/A

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:**

1. The LC_{50} was obtained from only 4 toxicant concentrations; although, initially, 5 were tested. Total mortality was observed at the lowest test level (13 µg ai/L) within the first 24 hours. The total mortality was attributed to the very low pH (3.6) measured at this concentration. No other similar pH readings were noted at the other test concentrations. Also, no mortalities or signs of toxicity were observed at the 24 and 33 µg ai/L concentration levels. From the data given, the mortalities observed at the lowest test concentration were not treatment related but due to the low pH.
2. The pH (excluding the lowest treatment concentration) of the dilution water (8.1-8.3) was greater than recommended (7.2-7.6).
3. The hardness of the dilution water (168 mg/L as $CaCO_3$) was greater than recommended (40-48 mg/L as $CaCO_3$).

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms:

Guideline Criteria	Reported Information
Species Preferred species is *Daphnia magna*	*Daphnia magna*
All organisms are approximately the same size and weight?	Not reported.
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3rd instar.	1st instar (≤24 h)

Guideline Criteria	Reported Information
Supplier	In-house cultures.
All organisms from the same source?	Yes

B. **Source/Acclimation:**

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Cultures maintained at conditions similar to test.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported.
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study.	No feeding.
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	Not reported.

C. **Test System:**

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water.	Hard blended well water.
Does water support test animals without observable signs of stress?	Yes
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20°C

Guideline Criteria	Reported Information
pH Prefer 7.2 to 7.6.	3.6 (at the lowest concentration level) and 8.1-8.3 (at all other treatment levels and control)
Dissolved Oxygen Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%.	≥89% during the test.
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$.	168 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 mL (daphnids and midges) or 2-3 L.	 Glass 1.6 L 1.0 L
Type of Dilution System Must provide reproducible supply of toxicant.	N/A
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.	N/A
Photoperiod 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.	None used.

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L, then no definitive test is required.	Yes, 10, 100, 1000, 10,000 and 100,000 µg ai/L. Immobilization >93% in the four highest treatment levels. No immobilization at the 10 µg ai/L test concentration.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Control, 13, 22, 36, 60, and 100 µg ai/L.
Number of Test Organisms Minimum 20/level, may be divided among containers.	20 per treatment, 10 per replicate.
Test organisms randomly or impartially assigned to test vessels?	Yes
Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	 Yes, measured continuously and daily by hand. Yes, measured every 24 hours.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Yes, at test initiation and termination.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Static: ≤10% Flow-through: ≤5%	0% mortality
Percent Recovery of Chemical	92-110%
Raw data included?	Yes

Mortality

Concentration (ppb ai)		Number of Organisms	Cumulative Number Dead	
			Hour of Study	
Nominal	Mean Measured		24	48
Control	<2.5	20	0	0
22	24	20	0	0
36	33	20	0	0
60	64	20	0	9
100	100	20	13	20

Other Significant Results: Total mortality was observed at the lowest concentration treatment level (13 µg ai/L) within the first 24 hours. The total mortality was attributed to the very low pH (3.6) measured at this concentration. No other similar pH readings were noted at the other test concentrations. Signs of toxicity (e.g., lethargy) were noted at the two highest-concentration treatment levels. No sublethal effects were observed at the 24 and 33 µg ai/L treatment levels.

B. Statistical Results

Method: Nonlinear interpolation

48-hr EC_{50}: 66 ppb ai 95% C.I.: 33-100 ppb ai
Probit Slope: N/A NOEC: 33 ppb ai

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC_{50} (C.I.)	66 (33-100) ppb
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	33 ppb ai

14. REVIEWER'S COMMENTS: This study is scientifically sound, fulfills the guideline requirements for an acute toxicity study using freshwater invertebrates, and can be classified as **Core**. The LC_{50} value of 66 ppb ai classifies didecyldimethylammoniumcarbonate as very highly toxic to *Daphnia magna*.

FEKEN DDAC DAPHNIA MAGNA 11-17-95

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	20	20	100	9.536742E-05
64	20	9	45	41.19014
33	20	0	0	9.536742E-05
24	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 33 AND 100 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 65.91389

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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